The frequency of OPRK1 G36T and OPRM1 A118G opioid receptor gene polymorphisms in heroin-dependent individuals and non-dependent healthy subjects in Turkey

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SUMMARY

Objective: Polymorphisms of the Mu opioid receptor (MOR) gene (OPRM1), which encodes for the primary action site of heroin, have also been found to be associated with heroin addiction. The aim of this study was to investigate the relationships between heroin addiction and G36T OPRK1 and A118G OPRM1 receptor gene polymorphisms in a male population in Turkey.

Method: 102 male patients with heroin use (without any other drug use) and 82 subjects without any history of opioid use were evaluated. The A118G and G36T SNPs on the MOR and Kappa opioid receptors (KOR) genes were assessed via TaqMan 5'-exonuclease allelic discrimination assays.

Results: The mean duration of heroin use was 4.6±1.9 years. The G36T polymorphism and heterozygous genotype were both found to be more frequent in the patient group (OPRK1 gene). In the patient group, 79 (77.5%) patients had wild-type genotype and 23 (22.5%) patients had mutant genotype. In the control group, 76 (92.7 %) subjects had wild-type genotype and 6 (7.3 %) subjects had mutant genotype (p=0.005). Wild type allele frequency was determined to be 0.894 and mutant type allele frequency was 0.105. With regard to the A118G polymorphism, we found that there was no difference between groups in terms of genotype.

Discussion: Our findings support a considerable role for OPRK1 in opioid addiction; however, in conflict with most studies, we did not determine a relationship with A118G in Turkish subjects. We suggest that further stu-dies should be conducted to ascertain the clinical implications of opioid gene polymorphisms in Turkey.

Key Words: Genetic, Dependence, Opioid, Polymorphism

INTRODUCTION

Addiction is a chronic relapsing condition caused by short- and long-term adaptations in the dopaminergic system, leading to all manners of change in epigenetic, mRNA, neuropeptide, neurotransmitter and protein levels (1). Heroin dependence (HD) is a chronic disease with medical, social and economic burden (2). It has been reported that the number of people using heroin has increased by 62.5% from 2002 to 2013 in the USA. The number of deaths related to high dose heroin use has dramatically increased by 285% in the same period (3). Turkey is geographically located in a region between drug producing and consuming countries, making it a transit country and a target for drug dealers.

DOI: 10.5505/kpd.2023.73693

Cite this article as: Demircan G, Toker Ugurlu T, Zengin G, Kepenek AO, Berk SC, Saygın D, Ozliman IN, Atesci F, Akin D. A The frequency of OPRK1 G36T and OPRM1 A118G opioid receptor gene polymorphisms in heroin-dependent individuals and non-dependent healthy subjects in Turkey. Turkish J Clin Psych 2023; 26: 163-169

The arrival date of article: 06.12.2022, **Acceptance date publication:** 01.05.2023

Turkish J Clinical Psychiatry 2023;26:163-169

Heroin directly interacts with opioid receptors (3). The opioid system is comprised of 3 types of G protein coupled receptors, mu (μ), kappa (κ) and delta (δ) (2). Single nucleotide polymorphisms (SNPs) in the opioid system have been shown to have significant effects on the tendency to develop addiction, and their frequencies change from population to population (4,5). There have been several studies showing that SNPs may influence HD and addictive characteristics. For instance, polymorphisms of the kappa receptor were associated with alcohol use and symptoms of withdrawal among recipients of methadone treatment (6,7). Kappa opioid receptors (KOR) play a modulatory role in the reward system by regulating dopaminergic activity. Whereas, presynaptic Mu opioid receptor (MOR) activity has been demonstrated to decrease Gamma aminobutyric acid (GABA) levels that inhibit the dopamine pathway (2,8). Chronic use of addictive drugs causes an upregulation in the KOR/dynorphin system, and the presence of the G36T SNP (located in exon-2) has been shown to be associated with addiction-related findings in humans and voluntary alcohol-drinking behavior in experimental animals (1,6). However, despite literature reviews yielding a high number of studies that found associations with the proclivity for dependence, there is also strong evidence to the contrary from different populations (9,10), which indicates the need for further population-based research on this subject.

Polymorphisms of the MOR gene (OPRM1), which encodes for the primary action site of heroin, have also been found to be associated with heroin addiction (11-14). In the OPRM1 gene, the presence of A118G SNP (located in exon-1) demonstrates a 3-fold increase in the affinity of MOR to beta endorphin; thus, A118G has gained interest due to its role in affinity, demonstrated by both in vivo and in vitro analyses (6). Furthermore, the rapid activation of MOR by heroin and/or analogues results in a euphoric effect, contributing to the development of drug addiction in humans, as suggested by previous studies (15).

In the light of this knowledge, our hypothesis is that heroin dependence is related to genes such as OPRM1 and A118G, and that genetic is a predictor for addiction.

Taken together, due to the lack of concrete data on this topic (whether or not these polymorphisms are indeed associated with dependency) and the apparent need for assessments in different populations (due to conflicting results), the aim of this study was to investigate the relationships between heroin addiction and G36T OPRK1 and A118G OPRM1 receptor gene polymorphisms in an outpatient population of the Alcohol and Substance Addiction Research, Treatment and Education Center (AMATEM) in Turkey. As far as we know, our study has the feature of being the first study to examine two different polymorphisms in the Turkish population after the reference study (16).

METHODS

Study Samples

In this study, 102 (55.4%) unrelated male patients who used only heroin were diagnosed with opioid use disorder according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria were included. Patients were recruited from the AMATEM clinic of Pamukkale University Department of Psychiatry. Approximately 500 patients applied to the AMATEM clinic between September and November 2019, when the data of the study were collected. Excluding non-heroin users, multiple substance users (excluding nicotine use), those who did not agree to participate in the study, female patients, and those with chronic psychiatric disorders (eg, mental retardation, psychosis, bipolar disorder…), the remaining 102 patients were included in the study. The inclusion criteria were as follows for the patient group: providing written informed consent, being aged older than 18 years, and recei-ving addiction therapy during the course of the study. No sampling method was used in the study, and all patients who were treated in the relevant clinic at the time of the study and who met the inclusion criteria and accepted to participate in the study were included in the study. Additionally, an age- and ethnicity-matched control group of 82 (44.6%) subjects without any history of opioid use were included. The control group was recruited from the same university employees. Declare that they have not used any substance in their lifetime

(excluding nicotine use), providing written informed consent, being male, being aged older than 18 years were the inclusion criteria for the control group. Participants with chronic psychiatric disorders (eg, mental retardation, psychosis, bipolar disorder…) were also excluded. All participants were resided in the same geographical area. They did not receive any monetary compensation for study inclusion and agreed to participate in the study voluntarily. Two Independent Proportions (Null Case) Power Analysis were performed on PASS 11 (Hintze, J. (2011). PASS 11. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com.). According to the power analysis, there should be minimum of 106 patients to detect the difference between the polymorphism rate of 75% and 59% with 0.05 alpha error and 80% power.

A questionnaire was used to record the sociodemographic and clinical characteristics of patients and controls. Opioid use disorder was diagnosed according to DSM-5 diagnostic criteria.

This study was carried out in accordance with the Helsinki Declaration and approved by Pamukkale University Medical Faculty Clinical Trials Ethical Committee (approval number: 10.09.2019/15). All subjects provided written informed consent before study inclusion.

DNA Isolation and Genotyping

Whole blood samples taken into ethylenediaminetetraacetic acid-treated tubes were used for DNA extraction with a commercially available kit (QIAamp DNA Blood Mini Kit Qiagen, Germany) according to the manufacturer's protocol. The DNA concentration was determined using a NanoDrop spectrophotometer (Thermo-Scientific, USA) and samples were stored at -20 °C until polymerase chain reaction (PCR) was performed.

The primers and the probes used in PCR were designed for the rs1051660 polymorphic region of OPRK1 gene and the rs1799971 polymorphic region of the OPRM1 gene. The gene sequence was found on https://www.ensembl.org/index.html database and primers were designed using the Primer 3 program. The sequences of designed primers were controlled with BLAST program (https://www.ncbi.nlm.nih.gov). Genotyping was carried out using the TaqMan 5'-exonuclease allelic discrimination assays (Bioline, SensiFAST™ Lo-ROX Genotyping) and Real-Time PCR system for the following variants: rs1051660 (G/T) in the OPRK1 gene and rs1799971(A/G) in the OPRM1 gene.

Statistical Analysis

All statistical analyses were conducted with the Statistical Program for Social Sciences (SPSS) for Windows (version 20.0) computer program. The Pearson's Chi Square and Fisher's Exact test were used to compare the distribution of categorical variables among groups. The results were evaluated with a 95% confidence interval (CI), and statistical significance was noted at a p-value of less than 0.05 ($p < 0.05$). Goodness of fit X2 test was used to assess deviations from the Hardy–Weinberg equilibrium (HWE) in the control group.

RESULTS

Sociodemographic Characteristics of the Sample

The study was completed with the participation of a total of 184 subjects, 102 patients with opioid use disorder and 82 healthy controls. All subjects were male and had a mean age of 23.4 ± 4.4 (18–40) years. Seventy-five patients (75%) were single, 22 (22%) were married and three (3%) were divorced/widowed. Three (3.2%) patients were illiterate, 19 were (20.4%) primary school graduates, 48 (51.6%) were secondary school graduates, 22 (23.7%) were high school graduates and one (1.1%) was a university graduate (Table 1).

Substance Use Releated Characteristics of the Patients

The substance use characteristics of the patients showed that 58 (56.9%) patients used heroin with inhalation, 42 (41.1%) used inhalation and intravenous injections (IV), and two (2%) used only intravenous injection. The average duration of drug use was 4.6 ± 1.9 (1–11) years. Anti-HCV

Table 1: Distribution of sociodemographics and heroin use-related characteristics in patients

	Variables	Patients	
		n	%
Marital status	Single	75	75
	Married	22	22
	Divorced/widowed	3	3
Education	Illiterate	3	3.2
	Primary school	19	20.4
	Secondary school	48	51.6
	High school	22	23.7
	University	1	1.1
Heroin Use	Inhalation	58	56.9
	Inhalation and intravenous injection	42	41.1
	Intravenous injection	2	2
Anti-HCV	Positive	17	16.7
	Negative	85	83.3
Treatment compliance during follow-up	Regular	30	29.4
	Irregular	28	27.5
	Lost to follow up	44	43.1

positivity was detected in 17 (16.7%) patients. Thirty (29.4%) of the patients visited the hospital regularly for follow-up studies, 28 (27.5%) visited irregularly and 44 (43.1%) patients never showed-up for follow-up (Table 1). Additionally, all patients were being treated with buprenorphine-naloxone.

OPRK1 G36T and OPRM1 A118G Opioid Receptor Gene Polymorphisms

The G36T polymorphism and heterozygous genotype were both found to be more frequent in the patient group (OPRK1 gene). In the patient group, 79 (77.5%) patients had wild-type genotype and 23 (22.5%) patients had mutant (heterozygous or homozygous) genotype. In the control group, 76 (92.7%) subjects had wild-type genotype and 6 (7.3%) subjects had mutant genotype (p=0.005) (Table 2). Wild type allele frequency was determined to be 0.894 and mutant type allele frequency was 0.105.

With regard to the A118G polymorphism, we found that there was no difference between groups in terms of genotype (Table 3).

In addition, polymorphisms (OPRK1 and A118G) in the patient group were compared according to anti-HCV, heroin use (inhalation and IV/inhalation) and treatment compliance during follow-up, and no significant difference was found (p>0.05).

DISCUSSION

Our results suggest an association between opioid addiction and the presence of G36T polymorphism in the OPRK1 gene; however, the A118G polymorphism results do not demonstrate any relationships, which is somewhat conflicting with previous studies. Nevertheless, it is apparent that various studies showing a relationship between drug addiction and OPRK1 gene polymorphisms (17-19) are supported by our findings. To our knowledge, this is one of the pioneering study investigating the association between heroin addiction and opioid gene polymorphisms (OPRK1 rs1051660 (G36T), OPRM1 rs1799971 (A118G)) in Turkey. Turkan et al. conducted a 103 patients included study which had investigated OPRM1 A118G polymorphism (rs1799971) in a Turkish population. They found that this polymorphism was associated with opioid and other substance addiction (16).

Since the function and features of opioid receptors make them obvious targets for addiction-related research, numerous studies have sought to identify

Table 2: Distribution of the rs1051660 (G36T) polymorphism in OPRK1 gene

	Patients		Controls		Overall		p*
	n	%	n	%	n	%	
Mutant (heterozygous+homozygous)	23	22.5	6	7.3	29	15.8	0.005
Wild-type (normal)	79	77.5	76	92.7	155	84.2	
Total	102	100	82	100	184	100	

*Pearson Chi-Square test, patients versus controls.

Table 3: Distribution of rs1799971 (A118G) polymorphism in OPRM1 gene

	Patients		Controls		Overall		p*
	n	%	n	%	n	%	
Mutant (heterozygous+ homozygous)	8	7.8	4	4.9	12	6.5	0.418
Wild-type (normal)	94	92.2	78	95.1	172	93.5	
Total	102	100	82	100	184	100	

*Pearson Chi-Square test, patients versus controls.

their exact roles via experimental and human-based investigations. Although exact mechanisms remain to be identified due to the complex relationships between the effects and overall functions of these receptors (20), all 3 types of receptors in the opioid system appear to have significant roles in addiction, be it through genetic, epigenetic or post-translational characteristics or adaptations. In this context, KOR seems to be a regulatory receptor due to its profound relationship with MOR effects (21), as well as demonstrating pro-addictive properties in the presence of anxiety and stress (22). For these properties, KOR has been suggested as a target for therapy in patients with addiction, especially in the presence of mood-related disorders (23). Although concluding any direct relationships with SNPs and the predisposition to HD would be far-fetched to say the least, our findings might be interpreted as being supportive in this context. That is, the presence of the G36T polymorphism in KOR may result in functional or efficacy-related changes in the receptor, possibly alleviating or eliminating its anti-MOR properties in patients with HD, which could explain the higher frequency of the G36T mutation in the patient group compared to controls. This hypothesis may find some support from the results of previous studies demonstrating a higher frequency of the G36T polymorphism in patients with HD (24,25). Furthermore, other alterations in the OPRK1 gene have also been associated with drug or alcohol abuse (26,27), indicating that further research must be performed to ascertain the mechanistic relationship between KOR and MOR in terms of addiction development (and its treatment).

It has been well established that MOR is the primary action site for opium derivatives (morphine, heroin, fentanyl and methadone) (4). Therefore, the gene encoding for human MOR is the main focus point of studies investigating the potential association between endogenous opioid system genes and alcohol and drug use (or addiction). The two common SNPs of the MOR gene (C17T and A118G), as well as the (CA)n repeat polymorphism have been studied in different ethnic, cultural and geographical populations (28). Ahmed et al. have shown a significant association between the A118G polymorphism and opioid addiction in Pakistanis (29). Tan et al. have demonstrated a significant difference between the allele frequencies of A118G SNP in an Asian population (30). The A118G SNP affects the β-endorphin binding affinity of MOR. Nucleotide 118 is the first base in codon 40 of the human MOR, and the A118G variant predicts an Asn-to-Asp change in amino acid residue 40 of the receptor (N40D) (31). It should also be noted that a remarkable study by Kumar et al. reported that the likelihood of both heroin and alcohol addiction were increased in the presence of A118G; whereas OPRK1 the three OPRK1 SNPs (rs16918875, rs702764 and rs963549) did not show any particular effect one way or the other. However, greater than 2-fold increases in the odds of having heroin and alcohol addiction was identified in the presence of specific alterations in OPRK1 (rs16918875) and OPRM1 (A118G) (27). This latter finding indicate an underlying relationship between the two receptors that could translate to increased susceptibility to opioid addiction.

A number of studies conducted on different populations have revealed a significant difference in the allele frequency of A118G polymorphism. As listed above, various studies report that OPRM1 gen polymorphisms are relevant to opioid dependence, but it is important to note that conflicting studies in different populations also exist, and their results are similar to ours. Although conflicting results on this topic are reported throughout the world (6,32,33), authors often suggest caution in the assessment and generalization of their results, since it is apparent that ethnic and racial differences may alter not only the frequency of SNPs, but also the exact effects/relationships they have with HD or other types of addiction.

In the patient group, both polymorphisms were found to be similar when compared according to anti-HCV positivity, using heroin intravenously or by inhalation and treatment compliance. In the literature, no data were found about the polymorphisms and hepatitis C in patients using opioids. OPRM1 and A118G were examined in terms of the effectiveness of methadone and buprenorphine

treatment, it was found that the efficacy was regulated in an allele-specific manner (34). In our study, all patients were using buprenorphine treatment. No difference was found in terms of treatment compliance compared to polymorphisms. In the study of Turkan et al. (16), alleles were found to be similar according to the history of psychiatric illness. In our study, chronic psychiatric diseases were excluded, but comorbid diseases such as depression, anxiety, attention deficit hyperactivity disorder were not considered.

There are several limitations of this study that must be discussed. The primary limitation is the lack of analysis for other established polymorphisms of the target genes; however, we were limited by available funding in this respect. Secondly, our study group was comprised of individuals who had sought treatment for HD; therefore, baseline characteristics of the participants may not be directly representative of the population of individuals with HD. The absence of substance use in the control group was accepted based on their statement and was not confirmed by any test (urine, etc.). Although the number for each group was 106 in the power analysis, the number of those who agreed to participate in the control group was 82. Lastly, genetic studies –and the relationships shown therein– must be supported by further analyses that demonstrate the alteration in protein levels and subsequent characteristics of patients –which is a limitation for all similar studies, as mentioned above.

To conclude, we showed that the OPRK1 (G36T) polymorphism frequency is significantly higher in heroin-dependent individuals compared to healthy control subjects comprised of males. It is critical to note that previous studies have suggested a role for sex-related differences in the function of these receptors (35,36); thus, it is inadvisable to predict similar findings among females. The OPRM1 (A118G) polymorphism frequency was not found to be at a higher frequency among heroin-dependent individuals in our study. It is recommended that future studies be planned with a larger sample size and to include the relationship of polymorphisms with treatment strategies, so that data can be correlated to the clinic practice.

Acknowledgement None.

Declaration of interest statement No potential conflict of interest was reported by the authors.

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